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Seroprevalence of Toxoplasmosis among children with Autism Spectrum Disorder in Mosul, Iraq

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ABSTRACT

Toxoplasmosis is regarded as one of the prevalent challenges in global health caused by parasite *Toxoplasma gondii*. Autism may be a single diagnosis, but the characteristics of autistic people are highly heterogeneous, include phenotypic traits, predictive factors, and biomarkers that contribute to the neurodiversity of autism, which include a variety of medical, social, ecological, and Indigenous models (and their combinations) of disability and difference. Autism The phenomenon known as Paraphraser has been recently associated with a variety of neuropsychiatric and behavioral conditions, with a particular focus on Autism Spectrum Disorder (ASD). The objective of this research endeavor was to assess the prevalence of Toxoplasmosis antibodies in children diagnosed with ASD.

Method. The investigation was carried out on 154 kids aged between 2 and 12 years, who were divided into two distinct groups. Group 1 comprised 94 children diagnosed with (ASD), while Group 2, serving as the control group, consisted of 60 healthy children. The diagnosis of ASD in the patient group was based on the criteria outlined in the DSM (Diagnostic and Statistical Manual of Mental Disorders). Each child partaking in the study underwent a thorough medical history assessment, a clinical test, and labs tests to identify serum anti-Toxoplasma IgM antibodies utilizing ELISA IgM. Furthermore, the patient group underwent additional evaluation using the Childhood Autism Rating Scale to assess the strong of their symptoms.

Results. The prevalence of IgM in children with ASD was found to be statistically insignificant when compared to that in typically developing children. There was no significant association observed between Toxoplasma infection and the strong of autistic symptoms in the ASD cohort. Moreover, the investigation indicated a higher level of anti-Toxoplasma IgM antibodies in ASD

children with a family history good of this disorder as opposed to those without such a history. Additionally, a higher distribution of anti-Toxoplasma antibodies was noted in children from low socioeconomic backgrounds in contrast to those from moderate or high socioeconomic backgrounds. The aim of the present study was to determine the seroprevalence of T. gondii infection in children with ASD in order to establish a potential link between toxoplasmosis and autism in children.

Conclusions. Conclusion: The current study revealed presence a high risk of getting autism after congenital toxoplasmosis but no definite clues was found. There are also higher infections of toxoplasmosis IgM in males compared to females.

Introduction

Autism is classified as a "spectrum disorder," The term "paraphraser" denotes a variation in the severity of symptoms displayed by a child. (ASD) comprises a collection of intricate neurodevelopmental conditions. Initial signs may include difficulties in social engagement, as well as verbal and non-verbal expression., as well as repetitive behaviors, are indicative traits of (ASD). Various factors, including levels of fetal testosterone, environmental influences, immunological disparities, obstetric complications, intrauterine infections, genetic predispositions, and gender disparities (with males exhibiting a higher prevalence of autism compared to females), have been associated with the causation of ASD [1]. A youngster with autism spectrum disorder may encounter challenges in effectively communicating and interacting with peers. This can manifest in the form of engaging in repetitive behaviors and movements, exhibiting frustration when faced with disruptions to their daily schedule, and displaying atypical responses to specific circumstances on occasion [2].

Toxoplasmosis is caused by *Toxoplasma gondii* Parasite Considered an epidemic disease that spreads widely worldwide, maternal infection in humans is estimated to affect between 30% and 50% of the global population [3]. The neonate acquires a significant portion of its IgM [4] antibody from the mother, making it a reliable indicator for maternal IgM levels. A study in Turkey revealed a Toxoplasmosis IgM positivity rate of 2.9% in the ASD group and 2% in the control group [5]. The genes of the T. gondii interactome showed a notable enrichment in the databases of susceptibility genes related to various diseases [6]. Fetal toxoplasmosis infection

disrupts the genes involved in the electron transport chain (specifically complexes I and III), which are crucial for the generation of free radicals and the production of oxidative stress. This interference contributes significantly to the pathophysiology of neurodevelopmental and neurodegenerative disorders like autism [7]. The majority of infected neonates are asymptomatic at birth but suffer vision problems later [8]. Symptoms of autistic disorder and indicators of development manifest. Preceding their offspring's initial year of life, caregivers have noted developmental apprehensions; however, as the child reaches two years of age, a significant proportion of caregivers express worries regarding linguistic progression and interpersonal engagement. [9]. *Toxoplasma gondi*i is Prevalence of the infection is widespread across various regions globally, reaching reported rates as high as 75%. Gender does not appear to significantly influence prevalence rates as individuals age. Moreover, the infection shows higher prevalence in environments characterized by high temperatures and humidity [10]. During the initial trimester of existence, premature neonates afflicted with toxoplasmosis could manifest central nervous system and ocular afflictions. Conversely, full-term neonates infected with T. gondii typically exhibit a less severe malady, characterized by hepatosplenomegaly and lymphadenopathy within the initial two months post-birth. Upon undergoing routine evaluations for newborns, a substantial proportion of the latter cohort—up to 80%—might subsequently experience cognitive or visual impairments [11].

Three primary classifications of Toxoplasma gondii genotypes exist, namely type I, II, and III. It is noteworthy that the Type II genotype predominantly contributes to the majority of congenital toxoplasmosis cases [12]. This study was aim to Investigate the epidemiology as a means of determining of Toxoplasmosis in children with ASD.

Material and Methods

A study involving (154) participants, (94) of whom were children with autism and (60) were healthy children between the ages of (2 – 12) years, was conducted in several Hospitals in Mosul, Iraq, from November 2023 to January 2024. Blood samples were taken from the children to test for Toxoplasma IgM levels using the ELISA technique.

Sampling: Initially, Ten ml of blood samples from (154) children (94 autistic patients and 60 control) using a sterile syringe were taken

Detection using ELISA Technique

Detection of Toxoplasma IgM using ELISA technique was performed by measuring anti-Toxoplasma IgG and IgM antibody levels using the Sandwich-ELISA method. The run utilizing the Sun Long Biotech . A purified toxo antigen was attached to a microplate according to the manufacturer's instructions in the laboratory. Samples were added to the plate wells and interacted with specific antibodies. Excess components were removed, and a substrate solution was applied, causing the wells containing toxo to change color from blue to yellow. The absorbance was measured at 450 nm, with higher values indicating higher concentrations of toxoplasma antigen. Concentrations were calculated by comparing the sample's absorbance to a standard curve, which allowed researchers to determine specific antibody levels in the serum.

Statistical Analysis. Descriptive statistics, as quantitative variables were described using mean and S.D. for normal distribution and median Qualitative variables were reported as numbers (percentages). Mann-Whitney and Kruskal-Wallis tests assessed IgG and IgM mean difference, false positive (+ve), and negative value, (-ve) values were calculated, determining accuracy indices (positive predictive value, specificity, sensitivity

Results and Discution

In the present study we enrolled (154) children, (94) with ASD and (60) with non- ASD as healthy control. The positive result was distribution in all ages, the cases with infection in male was higher than in female the age group for infection in males (2 – 4) years the S.D. was 0.178 , in (5 – 8) years the S.D. was = 0.289 and in (9 – 12) years the S.D. was= 0.331 while in (2 – 4) years female the S.D. was= 0.424 ,in 5 – 8 years the S.D. was = 0.424 and in 9 – 12 years was. D. = 0.303 while the P value was 0.0005 ** . the mean in males were 5.980 and in females were 7.179 of autistic cases as, while the P value was 0.045 as in Table 1 and 2.there was no significant differences between the values. The sepisifity was = 2.083 % and Sensefity was = 45.349 % as in Table 3

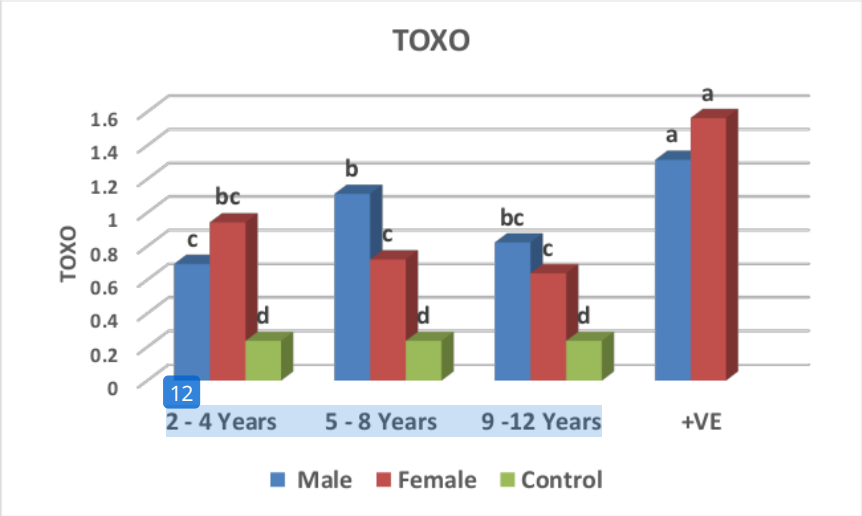

Figure 1: Distribution the Toxoplasmosis on autistic and control children According to Age Group in years

Table1: The Mean ± S.D of age group(years) of children with autism and control under study

Groups	No.	- VE Mean ± S.D.	+ VE Mean ± S.D.
Males	54	6.293 ± 2.791	5.980 ± 2.417
Females	40	8.090 ± 2.528	7.179 ± 2.812
Control	60	-	
P-Value		0.045 *	

Table 2: Mean ± S.D. of Seropsitivity against *Toxoplasma gondii* According to Age (Years)

Groups	AGE (Years)	No. -VE (94)	- VE Mean ± St.d	No.+VE (40)	+ VE Mean ± S.D.
Males	2 – 4	17	0.694 ± 0.169 e	8	1.314 ± 0.178 c
	5 – 8	29	1.111 ± 0.316 d	12	1.563 ± 0.289 b
	9 – 12	12	0.822 ± 0.211 de	6	1.586 ± 0.331 b
Females	2 – 4	4	0.941 ± 0.802 de	2	1.602 ± 0.424 ab
	5 – 8	14	0.721 ± 0.277 e	7	1.642 ± 0.199 ab

	9 – 12	22	0.639 ± 0.140 e	5	1.737 ± 0.303 a
control		60	0.236 ± 0.074 f	-	
P-Value		0.0005 **			

The same letters mean no difference between them. The dissimilar letters indicate that there is no significant difference between the groups for the age groups.

Table 3: Sensitivity and spesifity of ELIZA Technique under Study

Sensifity	45.349 %
SpecIfIcIty	2.083 %

An undeniable correlation exists between toxoplasmosis and various mental disorders such as depression, schizophrenia, and autism. Toxoplasmosis is a prevalent parasitic illness that affects humans as well as other endothermic organisms [13]. Studies have indicated that there WAS a notable prevalence of Toxoplasmosis infections among immunocompromised individuals and pregnant women [14]. In the context of pregnancy, infections, especially during the initial phases, have been associated with neurodevelopmental issues, predominantly ASD [15]. Serum levels of anti-Toxoplasma gondii IgM and IgG were quantified in children with (ASD) through Enzyme-Linked Immunosorbent Assay (ELISA) and were subsequently juxtaposed with those of healthy children within the corresponding age range. The results stem from an extensive meta-analysis that was undertaken. by Nayeri *et al* [16]. Toxoplasmosis has been associated with an elevated likelihood of autism, as evidenced by a greater seroprevalence of anti-*Toxoplasma gondii* IgG antibodies in individuals with autism in comparison to control cohorts. Moreover, *Toxoplasma gondii* has been posited as a potential risk element in the onset of autism [17] found a range of epidemiology of toxoplasmosis higher in autistic kids than in healthy individuals The study conducted by Hamid *et al*. [18] the seroprevalence toxoplasmosis in autistic children and normal kids. Children with autism had big rate of toxoplasmosis [19] the changes in epidemiology may resultant from variations in mothers' attributes, such as how they manageable their cats [20] their education history, their healthful living practices, and their feeding habits, as well as different in climatic conditions (rain, temperature, soil kinds, altitude, and dry weather) [21]. The findings of the presented study regarding a familial history of autism revealed that, when contrasted with children lacking such a history, children diagnosed with autism and possessing a positive familial background exhibited a notably elevated incidence of past toxoplasmosis. Nevertheless, no such marked distinction was observed in autistic kids with recent toxoplasmosis infection. This search in agreement to Baioumy *et al*. [22] who reported a significant contras between the seroprevalence of past and modern toxoplasmosis and a positive family background of other psychiatric disease which is schizophrenia among their study categories It show that the strong relationship between anti-Toxoplasma gondii IgG antibodies and the etiology of several

neuropsychiatric illnesses in general and autism in young children in particular play an important role with the need for more attention to the prenatal and postnatal screening of both mothers and their offspring. This could shed insight on the role of latent toxoplasmosis in the etiology of many neuropsychiatric disorders in mothers and their offspring. It is conceivable that toxoplasmosis plays a substantial role in the etiopathogenesis of mental health disorders, as posited by numerous scholars who have delineated diverse pathways by which Toxoplasma gondii parasites may impact the central nervous system [23]. The individual who declared that the impact of Toxoplasma gondii on the development of psychiatric disorders likely results from the immune response of the brain and the secretion of mediators such as interferon-gamma. Moreover, it was hypothesized that Toxoplasma gondii enhances dopamine levels and the activity of parasitic tyrosine hydroxylase, leading to an upsurge in the manifestation of anxiety [24].

Conclusion

The research findings indicated that past exposure to Toxoplasma in children, as opposed to recent exposure, might be associated with the development of (ASD). The Broader Autism Phenotype challenges could also be supported by biological studies of autism that include broader perspectives and topics, as well as enhanced communication and resources. the term “risk factor” a factor that heightens the likelihood of disease onset, the classification of autism as a disorder remains a topic of contention. Limited terminology options exist for the notion of "risk factors" in facilitating scientific comprehension of the origins, processes, or prognostic indicators of autistic characteristics within human cohorts. This factor may include many factors such microorganism such as parasites like Toxoplasma gondii, is obligate intercellular parasite that transport to the fetal from mother or by feed raw meat or contaminated food.

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Reference:

1- Gesundheit B, Rosenzweig JP, Naor D, Lerer B, Zachor DA, Procházka V, Melamed M, Kristt DA, Steinberg A, Shulman C, Hwang P. Immunological and autoimmune considerations of Autism Spectrum Disorders. Journal of Autoimmunity. 2013: 1:44:1-7.

2- Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. Current Opinion in Neurology. 2013 ;1;26(2):146-53.

3- Flegr J, Prandota J, Sovičková M, Israili ZH. Toxoplasmosis–a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. PloS One. 2014; 24;9(3):e90203.

4- Hill DE, Dubey JP. T*oxoplasma gondii*. Foodborne Parasites. 2018:119-38.

5- Esnafoglu E, Yancar Demir E, Cetinkol Y, Calgin MK, Erdil A, Yurdakul Erturk E, Dagli A. The seroprevalence of antibodies to *Toxoplasma gondii* among children with autism. Dusunen Adam Journal of Psychiatry and Neurological Sciences. 2017;30(4):309.

6- Hill, D E, Chirukandoth S and Dubey, J P. Biology and epidemiology of *Toxoplasma gondii* in man and animals. Animal Health Research Reviews, 2005;6: 41-61.

7- Al Malki JS, Hussien NA, Al Malki F. Maternal toxoplasmosis and the risk of childhood autism: serological and molecular small-scale studies. BMC pediatrics. 2021;21:1-9.

8- Sadeghi M, Riahi SM, Mohammadi M, Saber V, Aghamolaie S, Moghaddam SA, Aghaei S, Javanian M, Gamble HR, Rostami A. An updated meta-analysis of the association between *Toxoplama gondii* infection and risk of epilepsy. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2019 1;113(8):453-62.

9- Mainord M. The Presence of the Halo Effect in Individuals Diagnosed with Autism Spectrum Disorder and Neurotypical Individuals. 2022 p:1-15

10- Afshan K, Baseer S, Kiran S, Narjis G, Firasat S. Seroprevalence of Toxoplasma gondii in Pregnant and Non-Pregnant Women of Khyber Pakhtunkhwa, Pakistan. Pakistan Journal of Zoology. 2023 Oct 31;55(5):2173.

11- Smith NC, Goulart C, Hayward JA, Kupz A, Miller CM, van Dooren GG. Control of human toxoplasmosis. International Journal for Parasitology. 2021 Feb 1;51(2-3):95-121.

12- Assoni LC, Nakashima F, de Sousa VP, Paduan NJ, Andreasse IR, Anghinoni TH, de Faria Junior GM, Ricci Junior O, Castiglioni L, Brandão CC, de Mattos LC. Seroepidemiology of *Toxoplasma gondii* infection in blood donors in a population from the northwestern region of São Paulo state, Brazil. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2024;118(2):102-9.

13- Schumacher AC, Elbadawi LI, DeSalvo T, Straily A, Ajzenberg D, Letzer D, Moldenhauer E, Handly TL, Hill D, Dardé ML, Pomares C. Toxoplasmosis outbreak associated with Toxoplasma gondii-contaminated venison—high attack rate, unusual clinical presentation, and atypical genotype. Clinical Infectious Diseases. 2021 1;72(9):1557-65.

14- Goldstein EJ, Montoya JG, Remington JS. Management of Toxoplasma gondii infection during pregnancy. Clinical infectious diseases. 2008 ,15;47(4):554-66.

15- Prandota J, Elleboudy NA, Ismail KA, Zaki OK, Shehata HH. Increased seroprevalence of chronic toxoplasmosis in autistic children: Special reference to the pathophysiology of IFN-g and NO overproduction. International Journal of Neurology Research. 2015 ,1;1(3):102-22.

16- Al Malki JS, Hussien NA, Al Malki F. Maternal toxoplasmosis and the risk of childhood autism: serological and molecular small-scale studies. BMC pediatrics. 2021;21:1-9.

17- Nayeri T, Sarvi S, Moosazadeh M, Hosseininejad Z, Sharif M, Amouei A, Daryani A. Relationship between toxoplasmosis and autism: A systematic review and meta-analysis. Microbial Pathogenesis. 2020 Oct 1;147:104434.

18- Flegr J, Horáček J. Negative effects of latent toxoplasmosis on mental health. Frontiers in Psychiatry. 2020 18;10:495866..

19- Hamid N, Azizy B, Hamidynejat H. Comparison of the infection of Toxoplasma Gondii and aggression in autism and normal children. Sadra Medical Journal. 2020 ,21;8(3):249-62.

20- Esnafoglu E, Yancar Demir E, Cetinkol Y, Calgin MK, Erdil A, Yurdakul Erturk E, Dagli A. The seroprevalence of antibodies to Toxoplasma gondii among children with autism. Dusunen Adam Journal of Psychiatry and Neurological Sciences. 2017;30(4):309.

21- El-Sayed SH, Al-Shewy KA, Abdin EM, Hasan HM. Seroprevalence of toxoplasmosis among children with autism. The Egyptian Journal of Neurology, Psychiatry and Neurosurgery. 2024 8;60(1):42.

22- Agmas B, Tesfaye R, Koye DN. Seroprevalence of *Toxoplasma gondii* infection and associated risk factors among pregnant women in Debre Tabor, Northwest Ethiopia. BMC research notes. 2015;8:1-7.

23- Mohamed Sayed Baioumy A, Metwally Abo Alabbas M, El-Baz NA. TOXOPLASMOSIS AMONG SCHIZOPHRENIC PATIENTS. Al-Azhar Medical Journal. 2016 1;45(2):365-70.

24- Horacek J, Flegr J, Tintera J, Verebova K, Spaniel F, Novak T, Brunovsky M, Bubenikova-Valesova V, Holub D, Palenicek T, Höschl C. Latent toxoplasmosis reduces gray matter density in schizophrenia but not in controls: voxel-based-morphometry (VBM) study. The World Journal of Biological Psychiatry. 2012 1;13(7):501-9.

25- Etkin A, Prater KE, Schatzberg AF, Menon V, Greicius MD. Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. Archives of general psychiatry. 2009 , 1;66(12):1361-72.